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Evidence from animal studies indicates that ozone (O_3), the major component of environmental photochemical smog, depresses various intracellular hydrolytic enzymes and increases susceptibility to microbial infections. It would appear possible that O_3 induced alterations in normal leukocyte functions might underlie some of these reported alterations in human response to infectious agents. A study was designed to assess the effect of O_3 on peripheral blood leukocytes from 21 healthy young human males exposed to 784 $\mu g/m^3$ of ozone for 4 hr. The capability of polymorphonuclear neutrophils to phagocytize and kill microorganisms of respirable size was evaluated and phagocytic and bactericidal rates were determined. A significant decrease in intracellular killing was seen at 72 hr. postexposure ($p < 0.001$). A decrease in phagocytic ability was also noted at 72 hr ($p < 0.05$). These findings indicate that O_3 in low concentrations has a transient effect on leukocyte functions in humans. Such an effect may be mediated by alterations in cell membrane, opsonization functions, and/or interference with intracellular enzyme synthesis.

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Effect of Ozone on Leukocyte Function in Exposed Human Subjects[1]

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Evidence from animal studies indicates that ozone (O_3), the major component of environmental photochemical smog, depresses various intracellular hydrolytic enzymes and increases susceptibility to microbial infections. It would appear possible that O_3-induced alterations in normal leukocyte functions might underlie some of these reported alterations in human response to infectious agents. A study was designed to assess the effect of O_3 on peripheral blood leukocytes from 21 healthy young human males exposed to 784 $\mu g/m^3$ of ozone for 4 hr. The capability of polymorphonuclear neutrophils to phagocytize and kill microorganisms of respirable size was evaluated and phagocytic and bactericidal rates were determined. A significant decrease in intracellular killing was seen at 72 hr postexposure ($p < 0.001$). A decrease in phagocytic ability was also noted at 72 hr ($p < 0.05$). These findings indicate that O_3 in low concentrations has a transient effect on leukocyte functions in humans. Such an effect may be mediated by alterations in cell membrane, opsonization functions, and/or interference with intracellular enzyme synthesis.

INTRODUCTION

Ozone, a highly toxic and reactive gas and the major component of photochemical smog, has been considered to be a potentially important predisposing factor in respiratory infections (Bates, 1972; Goldsmith, 1968; Purvis *et al.*, 1961; Stokinger, 1965). Toxicological investigations have shown that the pathogenesis of O_3-induced enhancement in susceptibility to infection involves (a) an inhibition or suppression of intracellular hydrolytic enzymes of alveolar macrophages involved in phagocytosis and (b) digestion of inhaled foreign particles (Alpert *et al.*, 1971; Coffin *et al.*, 1968; Hurst *et al.*, 1970).

Most studies investigating the toxicity of O_3 have been focused on pulmonary effects since it was formerly believed that O_3 was destroyed, or neutralized, by the respiratory tract, and for this reason O_3 was not absorbed into the body. Only recently convincing evidence has accumulated that O_3 exposure produces extrapulmonary effects such a sphering of red blood cells following inhalation of 392 to 490 $\mu g/m^3$ (0.20 to 0.25 ppm) of O^3 for periods of 30 to 60 min (Brinkman *et al.*, 1964) and chromosome aberrations from exposures to 392 $\mu g/m^3$ (0.20 ppm) for 5 hr (Zelac *et al.*, 1971).

The U.S. Environmental Protection Agency has recently designed a comprehensive research program at the Clinical Laboratory Facility in Chapel Hill, North Carolina to evaluate the human health effects of controlled O_3 exposures

[1] These data were presented, in part, at the 104th Annual Meeting of the American Public Health Association, Miami Beach, Florida in October 1976.

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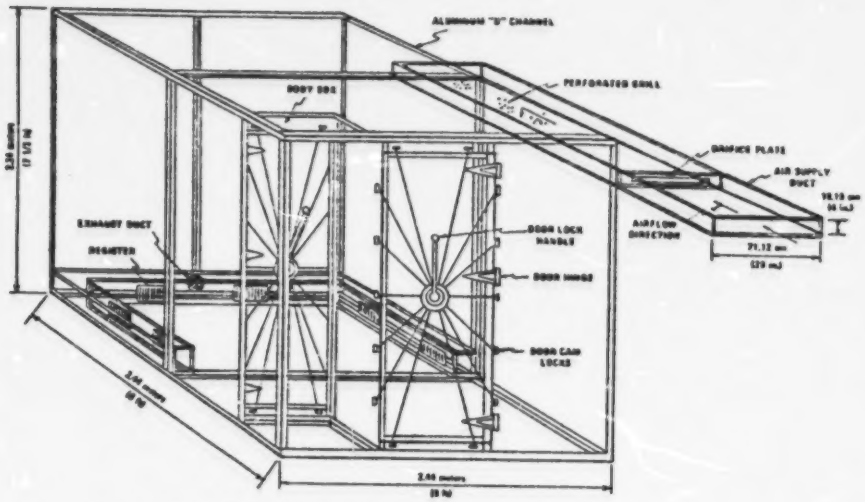


FIG. 1. Ozone and nitrogen dioxide human exposure chamber.

by measuring the most likely occurring clinical changes associated with known or suspected mechanisms of O_3 toxicity. A part of this research is concerned with measuring immunological changes affecting host defense mechanisms at the cellular level.

This report describes the effect of O_3 on the phagocytic and bactericidal abilities of leukocytes in the peripheral blood of humans exposed for 4 hr to 784 $\mu g/m^3$ (0.4 ppm) of O_3. The specific objective of this study was to measure the potential O_3-induced cellular alterations in the kinetics of the polymorphonuclear leukocyte (PMN) population that is an essential component of the immune defense system in human beings.

The results of this study provide evidence to indicate that human PMN leukocytes from O_3-exposed individuals exhibit an O_3-induced alteration not previously recognized, namely, a suppression of phagocytic and bactericidal activities.

MATERIALS AND METHODS

Subject selection. Male volunteers between the ages of 19 and 27 were studied after informed consent was obtained.[2] All subjects completed a comprehensive medical questionnaire and were examined by a physician. None of the subjects smoked or had a history of cardiac disease, allergy, or infection. No subject had used any medication for at least 1 week prior to testing.

Exposure protocol. Exposures to O_3 or clean air took place in an airtight 2.4 × 2.4 × 2.4-m chamber constructed from 1.3-cm thick acrylic plastic (Strong, *et al.*, 1977) (Fig. 1). Subjects were seated for 4 hr except for two 15-min exercise periods

[2] The protocol was approved by the Committee for Protection of Human Subjects of the University of North Carolina School of Medicine, Chapel Hill, North Carolina.

on a bicycle ergometer at a workload of 700 kg-m/min, an exercise level that produced an approximate doubling of heart rate. ECG was monitored continuously. The chamber was operated at a slightly negative pressure. O_3 was generated from oxygen (OREC O_3 generator) and injected into the ambient air-supply duct at a low-pressure point downstream from an orifice plate used to measure the flow of the ambient laboratory air into the chamber. The air–O_3 mixture was, after mixing, dispersed through a perforated grill. O_3 levels in the chamber were sampled continuously with two Bendix chemiluminescence analyzers. The concentration of O_3 was maintained between 686 and 823 $\mu g/m^3$ (0.35 and 0.42 ppm) at all times and was uniform in all parts of the chamber. During all exposures, ambient air was continuously monitored for O_3, sulfur dioxide, nitrogen oxides, carbon monoxide, and total suspended particulates. Concentrations of pollutants were extremely low at all times. Chamber temperature was maintained at 21 to 24°C and relative humidity was maintained at 40 to 60%.

Polymorphonuclear leukocyte suspensions. Ten milliliters of venous blood (with 20 ul/ml of heparin) was obtained from each of the subjects before each exposure, 4 hr after each exposure, and on Day 3 and 2 weeks after exposure to O_3. Polymorphonuclear neutrophils, separated by the method of Main and Jones (1968) and Silverman (University of North Carolina at Chapel Hill, personal communications), were washed twice with Hank's balanced salt–glucose solution (HBG) containing 0.01% bovine albumin fraction V (Cohn and Morse, 1959), counted in a hemocytometer, and resuspended. Cell suspensions so prepared contained an average of 52.3% PMN neutrophils (range, 32–90%) with a viability of greater than 98% on trypan blue exclusion. Red cell contamination was 2 to 4%.

Staphylococcus epidermidis served as the test bacteria. Overnight cultures grown in trypticase soy broth were washed twice in HBG and standardized to a known concentration (110–120 Klett units, 540 mμ) in a Klett–Summerson test tube model colorimeter to yield approximately 1 to 4 × 10^8 organisms/ml. An attempt was made to obtain an optimal bacteria-to-PMN neutrophil ratio of approximately 3 to 8:1 (Sbarra *et al.*, 1964); however, this was not always possible since the leukocyte yield at times decreased following exposure, resulting in a higher bacteria-to-PMN neutrophil ratio in the test system. The ratios, however, never exceeded 14:1, a ratio described elsewhere (Sbarra *et al.*, 1964).

Subject serum. Serum was obtained by allowing an additional 5 ml of blood from the subject to clot at room temperature for 30 min. The clot was rimmed and the serum was collected after centrifugation.

Experimental procedure. The experimental procedure for studying phagocytic and bactericidal rates has been previously described by Sbarra *et al.* (1964), Maaløe (1946), and Cohn and Morse (1959). The reactions were carried out in two series of siliconized sterile 25-ml size flasks consisting of 0.2 ml of *S. epidermidis* suspension of known concentration, 0.2 ml of autologous serum, and 0.6 ml of HBG. After 5 min equilibration in a water bath (37°C), 1.0 ml of the PMN neutrophile suspension was added to one series of flasks and 1.0 ml of HBG to the other series. The latter served as the bacteria–serum control. The flasks were placed on a rotating (circular rotation, 100 rpm) shaker in an incubator (37°C). At 30- and 60-min intervals, a 0.2-ml portion was removed from each of experimental and control flasks, diluted to 2 ml with trypticase soy broth, and homogenized with a

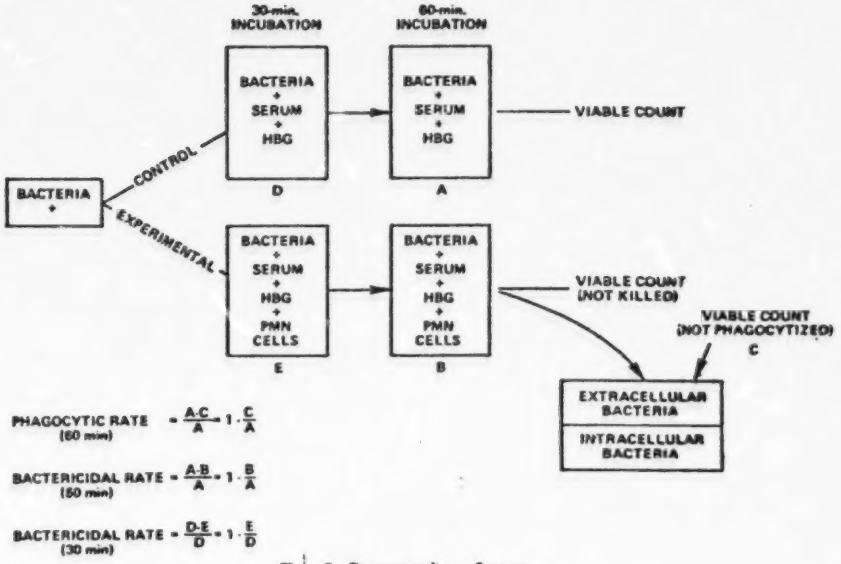


FIG. 2. Computation of rates.

Teflon homogenizer. The sample was then diluted in M/15 phosphate buffer and plated using the standard "pour plate" technique for viable cell counts. The procedure gave the total viable count. Next, at the 60-min incubation period, a 1.0-ml aliquot was removed from the experimental flask and added to 4.0 ml of HBG, mixed, and centrifuged at 500 rpm for 4 min in an International Model centrifuge (22°C). A 0.2-ml portion of the supernatant was removed, homogenized as before, diluted, and plated. This procedure gave the total extracellular bacterial count. The cellular pellet was then removed and suspended in 1 ml of HBG. A 0.2-ml portion of this was again removed, added to 1.8 ml of trypticase soy broth, homogenized, and plated for the total bacteria associated with the cells. All cultures were incubated at 37°C for 48 ± 3 hr. Bacterial colonies were counted using either the Quebec or the Electronic colony counters and were expressed as colony-forming units per milliliter. These data were then used to calculate phagocytic and bactericidal rates, as shown in Fig. 2.

Statistical analysis. For both the bactericidal and phagocytic rates, a one-way analysis of variance with repeated measurements was used to analyze the data. For each variable, three hypotheses were tested. The first was that there was no difference between the average rate before exposure and the average rate 4 hr after exposure. For the second and third hypotheses, average rates at 72 hr and 2 weeks were used instead of the 4-hr average rate.

RESULTS

Three days prior to O_3 exposure, the kinetics of neutrophil activity were studied in eight subjects before and after a 4-hr exposure to air in the chamber. The subjects followed the same exercise protocol as did O_3-exposed subjects. No statisti-

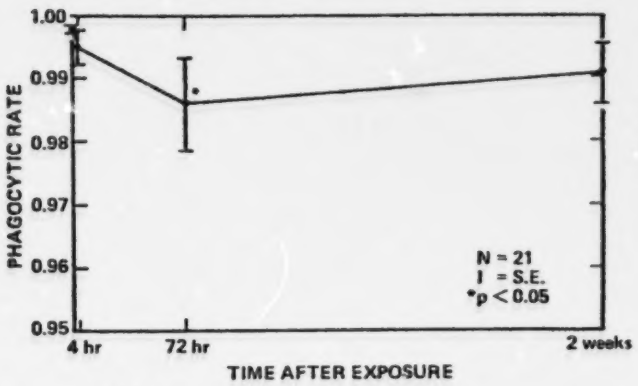


FIG. 3. Phagocytic rates (60-min incubation).

cally detectable differences were observed in mean phagocytic and bactericidal rates in samples taken before and immediately after the exposure.

Figure 3 illustrates the means ± standard error (SE) of the kinetics of phagocytic activity of neutrophils (incubated at 60 min) from 21 subjects before (0.998 ± 0.0005), 4 hr after (0.995 ± 0.0028), 72 hr after (0.986 ± 0.0072), and 2 weeks (0.991 ± 0.0048) after exposure to O_3. A statistically significant reduction ($p < 0.05$) of phagocytic capacity in samples taken at 72 hr following O_3 exposure was found. The impaired neutrophil function appeared immediately after the exposure and was again observed at 2 weeks; however, the mean rates obtained at these times were not statistically different from those at 0 hr.

Mean rates obtained on the kinetics of the bactericidal process following a 60-min incubation period are shown in Fig. 4. A significantly reduced bactericidal activity of neutrophils was also seen in samples at 72 hr (0.986 ± 0.0044 vs 0.997 ± 0.0010, $p < 0.001$). The bactericidal rates of samples obtained at 4 hr and

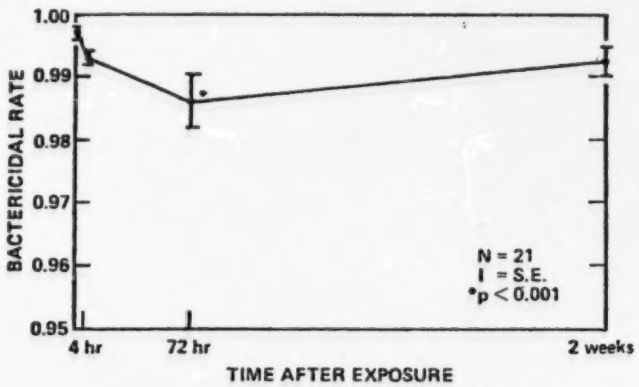


FIG. 4. Bactericidal rates (60-min incubation).

TABLE 1
COMPARISON OF PHAGOCYTIC RATES FOLLOWING 4-HR AIR AND OZONE EXPOSURES[a]

Time	Pre-air	Post-air	Pre-ozone	Post-ozone
Mean ($\bar{x}$)	0.999	0.996	0.997	0.997
S.E. ($\bar{x}$)	0.0004	0.0017	0.0009	0.0008

[a] $N = 8$.

2 weeks were not statistically different from the controls (0.993 ± 0.0012 for 4 hr and 0.993 ± 0.0023 for 2 weeks).

The nature of the defect was further characterized by studying both the phagocytic and killing abilities of neutrophils before and immediately after the 4-hr exposures to air and O_3. In these experiments, the phagocytic and bactericidal rates obtained for each postexposure were compared to the rates for "pre-air." As indicated in Table 1, the mean phagocytic rates from the eight subjects after air and O_3 exposures (0.996 and 0.997) were not statistically different from the pre-air value (0.999).

Data obtained on the kinetics of the bactericidal process before and immediately after air and 0_3 exposures are depicted in Table 2. The mean bactericidal rate observed at the 30-min incubation period after O_3 exposure was significantly reduced ($p < 0.05$) when compared to the pre-air value (mean, 0.986 for O_3 vs 0.995 for air).

However, the mean rate for post-O_3 at the 60-min incubation period (0.992), slightly reduced, did not differ statistically from the pre-air value (0.999).

COMMENT

Before the data from these experiments are evaluated, it is important to note that the current U. S. ambient air quality standard for photochemical oxidants is 160 $\mu g/m^3$ (0.08 ppm), maximum 1 hr concentration. The concentration of O_3 (784 $\mu g/m^3$) (0.4 ppm) used in these studies closely approximates O_3 levels found

TABLE 2
COMPARISON OF BACTERICIDAL RATES FOLLOWING 4-HR AIR AND OZONE EXPOSURES[a]

Thirty-minute incubation				
Time	Pre-air	Post-Air	Pre-ozone	Post-ozone
Mean ($\bar{x}$)	0.997	0.995	0.997	0.986[b]
S.E. ($\bar{x}$)	0.0007	0.0017	0.0011	0.0064
Sixty-minute incubation				
Time	Pre-air	Post-air	Pre-ozone	Post-ozone
Mean ($\bar{x}$)	0.999	0.998	0.999	0.992
S.E. ($\bar{x}$)	0.0005	0.0049	0.0006	0.0022

[a] $N = 8$.
[b] $p < 0.05$, from the analysis of variance.

on high smog days in certain areas of Southern California, where O_3 levels approaching 1176 $\mu g/m^3$ (0.6 ppm) for 1 to 2 hr have been measured (Monitoring of Air Quality Trends Report, 1973).

The mechanism of action of O_3 on peripheral blood neutrophils is, at present, open to speculation. The existing studies suggest that O_3 is radiomimetic and that the mechanism of its action may be in part through free radicals, a concept hypothesized for ionizing radiation (Brinkman and Lamberts, 1958). Many believe that the major pathway for the toxicity of O_3 involves oxidation of membrane-unsaturated fatty acids to produce ozonides, hydroperoxidases, and other long-lived free radicals (Buckley *et al.*, 1976; Goldstein and Balchum, 1967; Menzel *et al.*, 1972). These free radicals undergo chain reactions by which additional free radicals are produced, causing further cell damage. If such events occurred on PMN leukocytes, then phagocytic activity and intracellular structures involved in microbicidal events could be impaired. Such an effect may be mediated by alterations in cell membrane, opsonization (endocytosis) functions, and/or interference with intracellular enzyme synthesis.

As has been reported (Cline, 1974), a mature neutrophil has an average life span of 6.5 hr and it makes approximately 400 round trips through the vasculature before it is replaced. It appears that the delayed alteration in neutrophil function, such as that seen at 72 hr after O_3 exposure, results from O_3 interaction with lung tissue, which would attract circulating neutrophils, or O_3 or its active intermediates might be produced and transported through the vascular system. Ozone or its reactive byproducts may have altered the membrane receptors on the surface of the phagocyte, thus impairing bacterial attachment to the cell and subsequent stages of the phagocytic process. Normally, the phagocytic process is facilitated by certain humoral factors, such as (a) opsonins, which include immunoglobin G and M antibodies that bind specifically and aspecifically to surface constituents of the bacteria, and (b) heat-labile factors related to the complement and properdin system that further augments the process (Van Oss *et al.*, 1973). Any of these additional systems may have been altered by O_3.

The significantly reduced intracellular killing, such as that seen at 72 hr after O_3 exposure (Fig. 4 and Table 2), suggests the presence in the cells of free radicals or O_3 capable of altering the machinery responsible for enzyme induction. This possibility is supported by previous experimental observations showing an inhibition of enzymes of pulmonary cells in animals exposed to O_3 (Alpert *et al.*, 1971; Coffin *et al.*, 1968; Goldstein *et al.*, 1971; Hurst *et al.*, 1970). The assumption will need to be tested by determining the levels of intracellular enzymes and by analyzing metabolic reactions such as oxygen uptake and H_2O_2 reduction, which are important in microbicidal system of the cell.

In summary, the *in vitro* measurement of neutrophil functions in peripheral blood appears to be a sensitive method for determining phagocytic and bactericidal properties of PMN leukocytes in human beings exposed to air pollutants. Evaluations made to date indicate that changes observed in phagocytic and bactericidal functions correlate well with pathophysiological observations (Goldstein *et al.*, 1972; Goldstein *et al.*, 1974; LaForce *et al.*, 1971) and point to an intrinsic defect in neutrophils from human beings with microbial diseases or with neo-

plastic, immunologic, and other disorders (Koch, 1974; Sbarra *et al.*, 1964; Sølberg and Hellum., 1972).

The findings of this study provide new clinical evidence of O_3 effects not previously described in human beings and add new information on extrapulmonary O_3 toxicity.

REFERENCES

Alpert, S. M. Gardner, D. E., Hurst, D. J., Lewis, T. R., and Coffin, D. L. (1971). Effects of exposure to ozone in defensive mechanisms of the lung. *J. Appl. Physiol.* 31, 247–252.

Bates, D. V. (1972). Air pollutants and the human being. *Amer. Res. Respir. Dis.* 105, 1–13.

Brinkman, R., and Lamberts, H. B. (1958). Ozone as a possible radiomimetic gas. *Nature (London)* 181, 504–505.

Brinkman, R., Lamberts, H. B., and Veninga, T. S. (1964). Radiomimetic toxicity of ozonized air. *Lancet* 1, 133–136.

Buckley, R. D., Hackney, J. D., Clark, K., and Posin, C. (1976). Biochemical responses of humans to gaseous pollutants. Presented at the BEEP Symposium, Environmental Protection Agency. Cincinnati, Ohio.

Cline, M. J. (1974). Granulocytes in human disease. *Ann. Intern. Med.* 81, 801–816.

Coffin, D. L., Gardner, D. E., Holzman, R. S., and Wolock, F. J. (1968). Influence of ozone on pulmonary cells. *Arch. Environ. Health.* 16, 633–636.

Cohn, Z. A., and Morse, S. I. (1959). Interactions between rabbit polymononuclear leucocytes and stapylococci. *J. Exp. Med.* 110, 419–443.

Goldsmith, J. R. (1968). Effects of air pollution on human health. *In* "Air Pollution" (A. C. Stern, Ed.), pp. 547–615. Academic Press, New York.

Goldstein, E., *et al.* (1971). Ozone and the antibacterial defense mechanism of the murine lung. *Arch. Intern. Med.* 127, 1099–1102.

Goldstein, B. D., and Balchum, O. J. (1967). Effects of ozone on lipid peroxidation in the red blood cell. *Proc. Soc. Exp. Biol. Med.* 26, 356–358.

Goldstein, E., Eagle, M. C., and Hoeprich, P. D. (1972). Influence of ozone on pulmonary defense mechanisms of silicotic mice. *Arch. Environ. Health.* 24, 444–448.

Goldstein, E., Warshauer, D., Lippert, W., and Tarkington, B. (1974). Ozone and nitrogen dioxide exposure. *Arch. Environ. Health* 28, 85–90.

Hurst, D. J., Gardner, D. E., and Coffin, D. L. (1970). Effect of ozone on acid hydrolases of the pulmonary alveolar macrophage. *J. Reticuloendothel. Soc.* 8, 288–300.

Koch, C. (1974). Acquired defect in the bactericidal function of neutrophil granulocytes during bacterial infection. *Acta Pathol. Microbiol. Scand. Sect. B* 82, 439–447.

LaForce, M., Boehme, R., and Huber, G. (1971). Impaired pulmonary bactericidal activity in pulmonary edema. *Clin. Res.* 19, 324 (abstract).

Maaløe, O. (1946). "On the Relation between Alexin and Opsonin." Ejnar Munksgaards Forlag. Copenhagen.

Main, R. K., and Jones, M. J. (1968). Mixed leucocyte interaction involving mouse strains of strong and weak allogenic specificities. *Nature (London)* 218, 1251–1252.

Menzel, D. B., Roehm, J. N., and Lee, S. D. (1972). Vitamin E: The biological and environmental antioxidant. *Agr. Food Chem.* 20, 481–485.

Monitoring and Air Quality Trends Report. (1973). Publication No. EPA-450/1-74-007. Research Triangle Park, North Carolina.

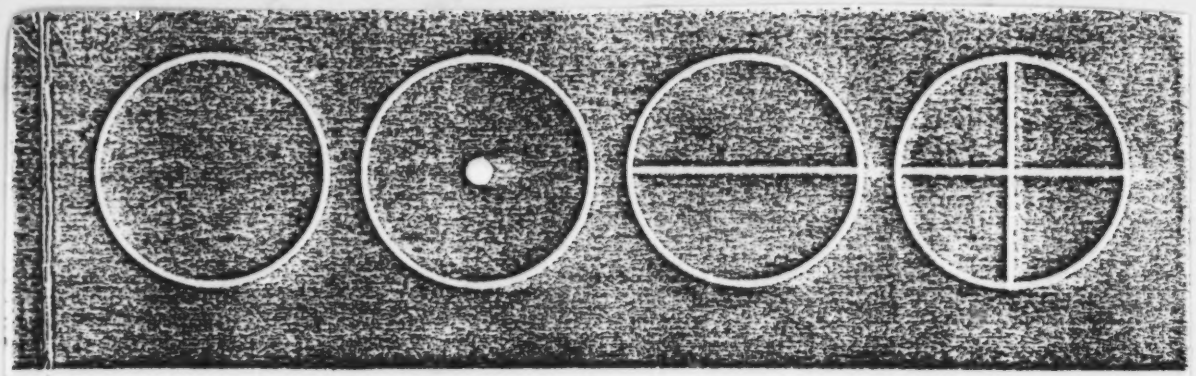
Purvis, M. R., Miller, S., and Ehrlich, R. (1961). Effect of atmospheric pollutants on susceptibility to respiratory infection. *J. Infec. Dis.* 109, 238–242.

Sbarra, A. J., Shirley, W., Selvaraj, R. J., Ouchi, E., and Rosenbaum, E. (1964). The role of the phagocyte in host–parasite interactions: I. The phagocytic capabilities of leukocytes from lymphoproliferative disorders. *Cancer Res.* 24, 1958.

Sølberg, C. O., and Hellum, K. B. (1972). Neutrophil granulocyte function in bacterial infection. *Lancet* 11, 727–730.

Strong, A., Penley, R., and Knelson, J. H. (1977). "Human Exposure System for Controlled Ozone Atmospheres." Publication No. EPA-600/1-77-048. Research Triangle Park, North Carolina.

Stokinger, H. E. (1965). Ozone toxicity. *Arch. Environ. Health* 10, 719–731.
Van Oss, C. J., Woeppel, M. S., and Marquart, S. E. (1973). Immunoglobulins as specific opsonins: III, The opsonizing power of fragments of polyclonal and monoclonal immunoglobulins G. *J. Reticuloendothel. Soc.* 13, 221–230.
Zelac, R. E., Cromroy, H. L., Bolch, Jr. V. E., Dunavant, B. G., and Bevis, H. A. (1971). Inhaled ozone as a mutagen: I, Chromosome aberrations induced in chinese hamster lymphocytes. *Environ. Res.* 4, 262–282.



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